progressive loss of the articular cartilage matrix due to an imbalance between matrix synthesis and degradation. Loss in the chondrocytes ability to respond to growth factor stimulation may be a key factor contributing to the development of OA and RA. We recently found that enforced expression of SOCS3 caused IGF-1 resistance in chondrocytes, thereby preventing upregulation of matrix proteoglycan synthesis. This study is designed to determine the expression of SOCS3 in human pathological chondrocytes and unravel the biological/functional consequences.
Methods: Chondrocytes were isolated from cartilage of patients undergoing surgical joint replacement. The mRNA and protein levels of SOCS1 and SOCS3 were measured by qPCR and Western blotting. Levels of SOCS3 were compared to a human immortalized chondrocyte cell-line (G6), mesenchymal-stem cells differentiated chondrocytes, and primary chondrocytes isolated from healthy bovine cartilage or from a SLE patient. The regulation of SOCS1 and 3 expression was studied in OA chondrocytes by incubation with different cytokines and TLR agonists. To determine the functional consequences the cytokine and TLR ligand-induced nitric-oxide production and IGF-1-stimulated proteoglycan (PG) synthesis was studied.
Results: The SOCS3 mRNA expression in articular chondrocytes and cartilage was markedly upregulated (32-fold) in 21/24 OA, 5/5 RA patients, 3/3 trauma patients tested, as compared to the G6 cell-line, stem cell-differentiated chondrocytes, and chondrocytes derived from a SLE-patient. Exposure of the mesenchymal stem cell-derived chondrocytes with conditioned media of OA synovial explants upregulated SOCS3 to the same extent as seen in the OA chondrocytes. Expression of SOCS3 could not be upregulated significantly in OA chondrocytes by the different cytokines (IL-1β, IL-17, IL-18) and TLR ligands (LPS, Pam2Cys, Poly(I:C), FK156, MDP) used. In contrast, SOCS1 expression was markedly lower (20-fold) in comparison to SOCS3 in OA chondrocytes and could be upregulated to the level of SOCS3 using IL-1β, IFNγ, IL-17, Pam2Cys, Poly(I:C), and especially with the combination of IFNγ and Poly(I:C). SOCS3 expression in OA chondrocytes was confirmed at the protein level. This means that at least in SOCS3 and SOCS1 genes are independently regulated and that SOCS3 has reached the maximal expression level. In the OA chondrocytes, the TLR4 ligand LPS was unable to induce NO production and IGF-1 failed to stimulate PG synthesis. Forced expression of SOCS3 in bovine cartilage-derived chondrocytes blocked the LPS (NO) and IGF-1 (PG-synthesis) response in these cells.
Conclusions: We found increased SOCS3 but not SOSC1 expression in human pathological chondrocytes. SOCS3 could block TLR4 and IGF-1 activation in chondrocytes. This suggests that SOCS3 modifies normal chondrocyte function and this could play a major role in cartilage pathology during arthritis.

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HIGH BODY MASS INDEX OF OSTEOARTHRITIS PATIENTS ASSOCIATES WITH SYNOVIUM INDUCED CHANGES IN CARTILAGE METABOLISM

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Purpose: Patient heterogeneity is probably one of the reasons for failure of osteoarthritis (OA) clinical trials. OA research has focused on cartilage destruction, while recent evidence shows other joint tissues, including adipose and synovial tissue, also play important roles in OA pathogenesis. Current literature indicates overweight is not only mechanically associated with OA, but also biochemically. Synovium modification leading to cartilage destruction is hypothesized to be a possible mechanism in OA development. We aim to investigate the influence of OA-derived synovium, in relation to body mass index (BMI), on cartilage metabolism.
Methods: Synovium of 16 OA and 8 healthy human donors with different BMI was cultured alone or together with bovine cartilage explants with or without stimulation by IL-1α. After 7 days, proteoglycan (PG) release, matrix metalloproteinase (MMP) activity and cytokines were measured. Cytokine profiles were subjected to multivariate analysis.
Results: Co-cultures of healthy or OA synovium with cartilage showed no differences in PG release without IL-1α stimulation. However, IL1-α stimulated co-cultures lead to a higher PG release from cartilage for OA compared to healthy synovium (54% vs 45% p=0.03). OA donors having BMI>30 induced a higher PG release then donors having BMI<30 (57% vs 48% p=0.006). With respect to MMP activity, no differences between OA and healthy synovium were observed. Multivariate analysis showed that synovium alone or in co-culture with cartilage can be distinguished based on their cytokine profile.
Conclusions: PG-release, a major process in OA development, is more prominent in co-cultures with OA-derived synovium compared to healthy synovium. BMI might prove to be a useful tool to stratify OA patient as synovium explants of high BMI OA donors lead to more cartilage degradation then synovium explants of low BMI OA donors. Interaction between synovium and cartilage leads to modification of cytokine profiles.

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INTERACTION OF HUMAN OSTEOARTHRITIC CARTILAGE AND SYNOVIAL TISSUE

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Purpose: Cartilage and synovial tissue are both affected in osteoarthritis (OA) and are known to interact. However, a long-term co-culture model with synovial tissue and cartilage explants has not been described before. Such a co-culture will provide a suitable model to study these interactions and mimic the OA joint more closely as well as to screen new therapies for cartilage degeneration in OA. Therefore, the aim of the study is to develop an in vitro model that includes both OA synovial tissue and cartilage, which can be used for testing new therapies for OA.
Methods: Osteoarthritic cartilage and synovial tissue were cultured together or alone for 21 days. To screen the effect of a therapeutic compound in the co-culture model, Triamcinolone 0.1mM was added to the culture media. To assess viability of the synovial tissue immunohistochemistry, a live/dead assay, and the release of lactate dehydrogenase (LDH) were used. Dimethylmethylene-blue assay was used to determine glycosaminoglycan (GAG) release and content of cartilage. Multiplex ELISA was used to determine the concentrations of secreted IL1, IL1-RA, IL4, IL6, IL7, IL8, IL10, IL13, TNF-alfa, oncostatin M, IFN-gamma, osteoprotegerin in the culture media.
Results: Throughout the entire culture synovial tissue showed viable cells by a LDH assay, a live/dead assay and immunohistochemistry demonstrated the presence of macrophages and T-cells. Several cytokines, which were previously demonstrated in synovial fluid of osteoarthritic patients, were secreted by synovial tissue during the entire culture period, indicating that the synovial tissue is still capable of producing cytokines during culture.
Co-culture of cartilage and synovial tissue enhanced GAG release and reduced GAG content after 21 days of culture compared to cartilage

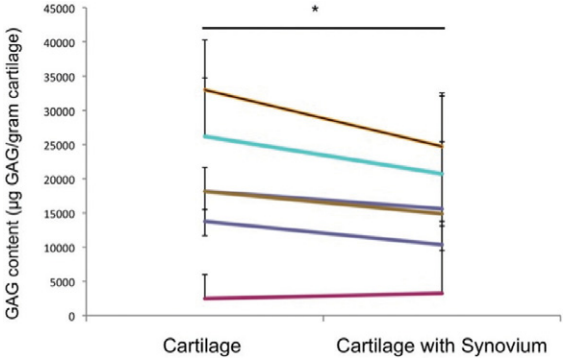

Figure 1. GAG content from 6 donors (mean ± SD in µg GAG/gram cartilage; *P<0.05).

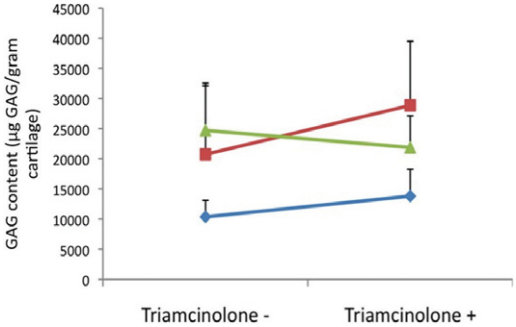

Figure 2. GAG content co-culture cultured with or without Triamcinolone (mean ± SD in µg GAG/gram cartilage).